

**AMENDMENTS TO THE SPECIFICATION**

Please amend paragraph 3 beginning at line 8 on page 7 with the following amended paragraph:

FIGS. 2A-2H are photographs showing NMDAR1 protein expression in lamina propria. Double label immunofluorescent staining combined with acridine orange counterstaining was used to visualize nuclei showed colocalization of NMDAR1 protein (FIG. 2B, and FIG. 2D, FIG. 2F, and FIG. 2H) with antibodies to gut cell markers (FIG. 2A) SIRP, and (FIG. 2C) dendritic cells, (FIG. 2E) helper T cells/macrophages, and (FIG. 2G) monocyte/macrophages;

Please delete paragraph 4 beginning at line 13 on page 7 and replace paragraph 5 at page 7 with the following amended paragraph as shown below:

~~—— Figs. 3A-3C are photographs of immunoblots of sera from AAVlac, AAVNMDAR1 and naïve control rats screened for the presence of  $\beta$ -galactosidase antibodies;~~

Figs. ~~3D-3H~~ 3A-3E are epitope map profiles of five different AAVNMDAR1-treated animals (N11, N19, N21, N52, and N64). Specificity was measured as a ratio between the AAVNMDAR1 signal and mean AAVlac signals for each peptide;

Please delete paragraph 8 beginning at line 22 on page 7:

~~Figs. 4B-4K show high power images of neurons to analyze hippocampal damage using fluorescent TUNEL labelling (Figs. 4B,D,F,H,J) or clusterin immunohistochemistry (Figs. 4C,E,G,I,K) combined with immunohistochemistry with NeuN, a mature neuronal marker;~~

Reply to Notice of Non-Compliant Amendment of Sept. 12, 2007 and  
Supplemental Reply to Office Action of November 30, 2006

Please amend paragraph 7 beginning at line 27 on page 8 with the following amended paragraph as shown below:

~~Figs. 8A-8I and Figs. 8K-8M are confocal images showing intracellular  $\text{Ca}^{2+}$  imaging of cultured mesencephalic neurons and AAVNMDAR1 IgG inummoreactivity in mesencephalic and rat hippocampal neurons. Fig. 8J 8 is a bar chart showing the ratio of changes in fluorescent intensity relative to basal levels between AAVlac and AAVNMDAR1 IgG-treated cells;~~

Reply to Notice of Non-Compliant Amendment of Sept. 12, 2007 and  
Supplemental Reply to Office Action of November 30, 2006

Please delete paragraphs 1-10 on page 9 as shown below:

~~Figs. 9A-9L are autoradiographs demonstrating NMDA receptor upregulation in the hippocampus of AAVNMDAR1-vaccinated animals using the three markers of NMDA upregulation;~~

~~Figs. 9A-9B are <sup>3</sup>H-MK-801 autoradiographs;~~

~~Figs. 9C-9D show NMDAR1 immunohistochemistry with a commercial antibody;~~

~~Figs. 9E-9F shows the results of *in situ* hybridization with NMDAR1 oligonucleotide probes;~~

~~Figs. 9G-9H shows the results of *in situ* hybridization with NMDAR2a oligonucleotide probes;~~

~~Figs. 9I-9J shows the results of *in situ* hybridization with and NMDAR2b oligonucleotide probes showing increased binding, immunoreactivity and mRNA levels in AAVNMDAR1-vaccinated animals (Figs. 9B,D,F,H,J) compared to AAVlac-treated animals (Figs. 9A,C,E,G,I);~~

~~Figs. 9K-9L show hippocampal mRNA levels of the trkB receptor were not significantly different in AAVlac-treated (Fig. 9K) and AAVNMDAR1-vaccinated animals (Fig. 9L).~~

~~Figs. 10A-10I are immunohistochemical images demonstrating antibody passage across an intact blood-brain barrier and the reduction of basal levels of Krox-24 protein within the cortex of AAVNMDAR1-vaccinated animals (Fig. 10C) compared to AAVlac-treated (Fig. 10B), or naïve animals (Fig. 10A). Insets show high-powered images of Krox-24 immunoreactivity;~~

~~Fig. 10I shows an immunoblot analysis of the cerebrospinal fluid (CSF) sampled from an AAVlac-treated animal (Lane 1) and AAVNMDAR1-vaccinated animal (Lane 2). A 117 kDa protein band corresponding to NMDAR1 receptor subunit was identified. Increased levels of the 117 kDa protein was found 90-min following kainate treatment (Lane 3);~~

~~Figs. 11A-11I are immunohistochemical images demonstrating the lack of inflammatory responses in the brain associated with vaccination;~~

Please replace paragraphs 1-6 at page 10 with the following amended paragraphs:

Fig. ~~12A~~ 9A demonstrates errors and latencies recorded on the Barnes Circular Maze test. Data represents the number of line crossings in 5 min intervals over 5 successive days in AAVlac (triangle-solid line) or AAVNMDAR1 rats (squares-solid line);

Fig. ~~12B~~ 9B demonstrates the line crossing and circular track mobility test in AAVlac (squares -solid line) or AAVNMDAR1 rats (triangle -solid line);

Fig. ~~12C~~ 9C demonstrates the data from the contextual fear conditioning test for AAVlac-treated (narrowly spaced upward sloping lines) and AAVNMDAR1-vaccinated animals (widely spaced downward sloping lines) (\* $p=0.025$ );

Fig. ~~12D~~ 9D demonstrates the data from the Spontaneous Object Recognition test in control (solid white) and AAVNMDAR1-vaccinated animals (narrowly spaced upward sloping lines);

Fig. ~~13~~ 10 is a bar chart demonstrating the effect of vaccination with AAVNMDAR1 on nociception. The latency for escape responses for the tail immersion test, and the latency for escape responses or hindpaw licking in the hot plate test for AAVlac-treated (black bars), and AAVNMDAR1-vaccinated (white bars) animals. Each bar represents the mean  $\pm$  SEM for all animals in that group (\*  $p=0.04$  for tail immersion and  $p=0.02$  for hot plate tests, Student's t-test).

Please replace the paragraphs beginning at page 56, line 10 and ending on page 58, line 12 with the following amended paragraphs:

(iii) Circulating antibodies

To determine the presence of circulating NMDAR1 antibodies, blood was removed from the vaccinated animals and the serum analyzed for presence of specific antibodies. ~~Fig. 3A shows the results from sera~~ Sera (1:200) from AAVlac, AAVNMDAR1 and naïve control rats, were screened by immunoblot analysis for the presence of  $\beta$ -galactosidase antibodies. 1  $\mu$ g purified  $\beta$ -galactosidase was loaded per lane (gel not shown). (Lane c, commercial  $\beta$ -galactosidase antibody; Lane 1, naïve control serum; Lane 2, AAVNMDAR1 serum 4 weeks after vaccination; Lane 3, AAVlac serum 4 weeks after vector administration; Lanes 4-5, AAVlac serum from two individual animals 4 months after vector). The results show that in AAVlac-treated animals, IgG antibodies were detected at 4 weeks with titres further increasing at 16 weeks. Using a purified beta-galactosidase enzyme preparation, a commercial monoclonal antibody recognized two molecular weight protein species of 116 and 85kD on immunoblots (~~Fig. 3A~~). Some animals had antibodies that bound preferentially to the 85kD species whereas other animals had serum antibodies with higher affinity to the 116kD protein (~~Fig. 3A~~).

~~Fig. 3B shows the results from serum~~ (1:200) from AAVlac, AAVNMDAR1 and naïve control rats, screened by immunoblot analysis for the presence of NMDAR1 antibodies. 20  $\mu$ g hippocampal membrane extract was loaded per lane. (Lane c, commercial NMDAR1 antibody (Chemicon, MAB363); Lane 1, naïve serum; Lane 2, AAVlac serum (4 weeks); Lane 3, AAVNMDAR1 serum (4 weeks); Lane 4, AAVNMDAR1 serum (4 months)). The results showed that immunoblotting of AAVNMDAR1 serum to a hippocampal membrane extract yielded the expected 117kD band, consistent with the molecular weight of the native NMDAR1 receptor subunit and similarly recognized by commercial antibodies. The sera from naïve control (n=4) or AAVlac (n=12) immunized animals showed no binding to the brain extract.

~~Fig. 3C shows the results from sera~~ Sera (1:200) were screened against a denatured hippocampal extract, 20  $\mu$ g was loaded per lane. (Lane P, polyclonal NMDAR1 antibody (Chemicon AB1516); Lane M, monoclonal NMDAR1 antibody (Chemicon, MAB363); Lanes

N35, N64, N52, is AAVNMDAR1 serum from three different animals showing specific affinities for different NMDAR1 breakdown products. (gel not shown) Brain extracts prepared under more severe denaturing conditions resulted in degradation of the native receptor as shown by detection with a commercial monoclonal antibody which bound fragments running at 32kD and 67kD. Of interest, individual AAVNMDAR1 rats showed different patterns of binding to some of these fragments (Fig. 3C-Lanes N35, N64 and N52). These data demonstrate that AAV can serve as an oral vaccine and that immunized animals generated antibodies against a range of NMDAR1 epitopes.

(iv) Epitope mapping of NMDAR1

To further define the range of epitopes, a total of 94 overlapping 16mers were synthesized covering the entire 938 amino acids of the native NMDAR1 protein as described in Example 1K. Serum from AAVNMDAR1, AAVlac and naïve rats were screened against this panel of 16mers.

Figs. ~~3D-H~~ 3A-3E of rats N11, N19, N21, N52, N64 show the epitope map profiles of five different AAVNMDAR1-treated animals. Specificity is measured as a ratio between the AAVNMDAR1 signal and mean AAVlac signals for each peptide. The results showed that none of the naïve (n=4) or AAVlac rats (n=14) screened had specific binding to any of the peptides. In contrast, AAVNMDAR1-immunized rats showed specific binding to peptides which corresponded to functional domains within the extracellular segments of the receptor. These included peptide 49 (amino acids 483-498) which represented the N-terminal side of M1, the first transmembrane domain (rat N19, Fig. ~~3E~~ 3B), and two peptides corresponding to the extracellular domain between M3 and M4, peptide 69 (amino acids 681-696; rat N21, Fig. ~~3F~~ 3C) and peptide 72 (amino acids 711-726; rat N64, Fig. ~~3H~~ 3E). Each of these three peptides contain critical residues for glycine binding (Kuryatov *et al* (1994) *Neuron* 12: 1291; Wafford *et al.* (1995) *Mol. Pharmacol.* 47: 374; Wood *et al.* (1997) *J. Biol. Chem.* 272: 3532). The most common pattern observed in serum from 7 of the 19 AAVNMDAR1 rats screened was specific binding to peptides adjacent to the M4n region (peptide 80, amino acids 791-807) and/or peptide 65 (amino acids 641-657) corresponding to the M3c domain (rat N11, Fig. ~~3D~~ 3A). Two additional rats had antibodies that bound to peptides 54/55 (amino acids

541-566; rat N52, Fig. 3G 3D) which mapped to the preM1 domain. The preM1, M4n and M3c regions form part of the extracellular vestibule of the NMDA receptor channel where amino acid substitutions at key residues have a significant influence on channel permeability (Beck *et al.* (1999) *Neuron* 22: 559).

Please replace the paragraphs from line 30 on page 59 through line 24 on page 60 with the following amended paragraphs:

~~Figs. 4B, D, F, H, and J show the analysis~~ Analysis of hippocampal damage using fluorescent TUNEL labelling, ~~while Figs 4C, E, G, I and K show the analysis of hippocampal damage and~~ using clusterin immunohistochemistry combined with immunohistochemistry with NeuN, a mature neuronal marker. As shown in the hilar region, all AAVlac animals (~~Figs. 4B, C~~) that developed SE showed numerous TUNEL-positive (arrows) and clusterin-immunofluorescent neurons, indicative of extensive neuronal death in the hippocampus. No TUNEL or clusterin immunofluorescence was found in any AAVNMDAR1-vaccinated rat that did not have seizures (~~See Figs. 4D, 4E~~).

One AAVNMDAR1-vaccinated rat that developed SE also showed extensive clusterin (~~Fig. 4G~~) and TUNEL staining (~~Fig. 4F~~). Of interest, the second animal that developed SE showed no TUNEL signal (~~Fig. 4H~~) or clusterin immunofluorescence (~~Fig. 4I~~). The EEG recordings shown in (Fig. 4A) correspond to the brains analyzed. ~~shown in (Fig. 4B-4I).~~ Kainate-induced seizures were also elicited in AAVGAD65-vaccinated animals and TUNEL (~~Fig. 4J~~) and clusterin labelling (~~Fig. 4K~~) confirmed extensive neuronal damage in the hippocampus. Scale 200  $\mu$ m.

The results demonstrated that no discernible TUNEL signal or clusterin immunofluorescence (a cell death marker, *see Dragunow et al. (1995) Mol. Brain Res. 32: 279*) was observed in the hippocampus of any animal (AAVlac, naïve or AAVNMDAR1) that did not develop SE (~~Fig. 4D, E~~). In comparison, all AAVlac and naïve animals that experienced SE had numerous TUNEL-positive and clusterin-immunofluorescent neurons in hippocampal CA1, CA3 and hilar regions (~~Fig. 4B, C~~). Of the two AAVNMDAR1 animals that had SE, only one of these animals (rat N12) had some injury (~~Fig. 4F, G~~), whereas the second animal (N7) had no injury whatsoever, despite over 45 minutes of severe SE (~~Fig. 4H, I, Table 1~~).



Please replace the paragraphs beginning at line 11 on page 67 and ending at line 27 on page 71 with the following replacement paragraphs:

The results from these studies are described below. ~~shown in Figs. 8A-8M.~~ Fig. 8A shows confocal Confocal images of non-treated control cells were taken. Confocal images were taken showing intracellular  $\text{Ca}^{2+}$  imaging of cultured mesencephalic neurons and AAVNMDAR1 IgG inummoreactivity in mesencephalic and rat hippocampal neurons. ~~Fig. 8D shows AAVlac IgG-treated cells, Fig. 8G shows NMDAR1 IgG-treated mesencephalic cells~~ preloaded with the  $\text{Ca}^{2+}$  indicator Oregon Green 488 BAPTA-1 (2  $\mu\text{M}$ ) showed low level fluorescence. Cells were preincubated with 50  $\mu\text{g}/\text{ml}$  IgG for 16 h prior to indicator loading. In response to a 100  $\mu\text{M}$  NMDA + 3 pM glycine challenge, the increase in fluorescent signal found in non-treated control (Fig. 8B) and AAVlac IgG-treated cells (Fig. 8E) was significantly attenuated in AAVNMDAR1 IgG-treated cells (Fig. 8H). Images are pseudocoloured according to fluorescent intensity, with transition from red to yellow representing basal  $\text{Ca}^{2+}$  levels to higher  $\text{Ca}^{2+}$  concentrations. Fig. 8J shows ratio of the changes in fluorescent intensity relative to basal levels showed a significant difference between AAVlac and AAVNMDAR1 IgG-treated cells. Each bar represents the mean  $\pm$  SEM, n=10 (\*p=0.0012, Student's t-test). Anti-rat IgG immunocytochemistry showed only purified AAVNMDAR1 IgG (Fig. 8I) bound to mesencephalic cells and not AAVlac IgG (Fig. 8F) which exhibited basal immunoreactivity similar to non-treated cells. (Fig. 8C) Using the IgG fractions to perform immunohistochemistry on brain sections, as shown in hippocampal hilar neurons, the AAVNMDAR1 IgG (Fig. 8L) showed a pattern of immunoreactivity similar to that found with a commercial NMDAR1 polyclonal antibody (Fig. 8M), while AAVlac IgG (Fig. 8K) showed only low level background immunoreactivity. Scale for Figs. 8A-H was 20  $\mu\text{m}$ , for Figs. 8C, F, I, was 30  $\mu\text{m}$ , and for Figs. 8K-M, was 100  $\mu\text{m}$

The results show that in untreated cells, or cells incubated with IgG from AAVlac rats (n=10) at a concentration of 50  $\mu\text{g}/\text{ml}$ , a marked increase in the fluorescent signal was obtained following NNMA application (~~Figs. 8A,B,D,E~~). Mesencephalic neurons incubated with the same concentration of IgG purified from AAVNMDAR 1-immunized rats (n=10) blocked the increase in intracellular calcium (~~Figs. 8G, H, J~~). Confirmation of specific

binding was obtained by immunocytochemistry with a secondary anti-rat IgG which was applied to untreated primary mesencephalic cells or cells that had been incubated with AAVlac or AAVNMDAR1-purified IgG (~~Figs. 8C,F,I~~). The results showed that only IgG purified from AAVNMDAR1 rat serum bound to the primary neuronal cultures (~~Fig. 8I~~). These results demonstrate that the antibodies can directly modulate the function of the NMDA receptors.

(ii) Self-Antigen Recognition

To demonstrate the recognition of self-antigens by the serum of AAVNMDAR1-vaccinated rats, immunoglobulins were purified using protein G columns and used as the source of primary antibody for immunohistochemistry of rat brain sections (*see* Example 1D and 1G). Sections at the level of the hippocampus were selected, because NMDA receptors are highly expressed in this region. Purified IgG fractions from AAVlac rats showed low level background immunoreactivity in control hippocampal sections (~~Fig. 8K~~). In contrast, IgG purified from AAVNMDAR1 rats showed specific signals in CA1, CA3 and the hilus (~~Fig. 8L~~), consistent with previous reports on NMDAR1 immunoreactivity in the rat brain and similar to the pattern we observed with a commercial NMDAR1 polyclonal antibody (~~Fig. 8M~~).

(iii) Transport of antibodies across the blood-brain barrier and NMDA receptor up regulation.

Transport of the antibodies across the blood-brain barrier and subsequent binding to the native receptor on brain parenchyma tissue was investigated at basal physiological conditions. Groups of AAVlac (n=7) and AAVNMDAR I rats (n=7) were euthanised 4 weeks after peroral dosing of the vaccine and the brains were removed and frozen.

<sup>3</sup>H-MK-801 autoradiography was used to label the open NMDA receptor channel in hippocampal sections using the technique described by Huettner *et al.* (Huettner *et al.* (1988) *Proc. Natl. Acad. Sci. U. S. A.* 85: 1307-1311) (*see* Example 1O). Three markers of NMDA receptor binding were studied. ~~are shown in Fig. 9A-9C.~~ These include <sup>3</sup>H-MK-801 autoradiography (~~Figs. 9A, B~~), NMDAR1 immunohistochemistry with a commercial antibody (~~Figs. 9C, D~~) and *in situ* hybridization with NMDAR1 (~~Figs. 9E, F~~), NMDAR2a (~~Figs. 9G, H~~) and NMDAR2b (~~Figs. 9I, J~~) oligonucleotide probes showed increased binding,

immunoreactivity and mRNA levels in AAVNMDAR1-vaccinated (~~Figs. 9B, D, F, H, J~~) compared to AAVlac-treated hippocampus (~~Figs. 9A, C, E, G, I~~) suggestive of NMDA receptor upregulation. In contrast, hippocampal mRNA levels of the trk B receptor (~~Figs. 9K, L~~) in AAVlac (~~Fig. 9k~~) and AAVNMDAR1 (~~Fig. 9L~~) treated rats were not significantly different. The results showed an increase in  $^3\text{H}$ -MK-801 binding (arrows) in the hippocampus of AAVNMDAR-vaccinated rats (~~Figs. 9A, B~~). Relative density measurements from film autoradiograms showed there was a significant increase in soma and dendritic binding in the CA1 hippocampal region of AAVNMDAR1 compared to AAVlac animals ( $0.281 \pm 0.006$  vs  $0.2580 \pm 0.008$ ;  $p=0.049$ , Student's t-test) while there was no significant difference in binding in the dentate granule cell or CA3 layer.

A commercial NMDA receptor-specific antibody was used to determine whether increased  $^3\text{H}$ -MK-801 binding was associated with an increase in NMDAR 1 protein expression. NMDAR1 rats had a significantly higher level of NMDAR1-immunoreactivity in the hippocampus ( $0.342 \pm 0.005$  vs  $0.314 \pm 0.002$ ,  $p=0.0009$ , Student's t-test) (~~Figs. 9C, D~~). Moreover, *in situ* hybridization using probes to NMDAR1, NMDAR2A and NMDAR2B subunits and density measurements of film autoradiograms also showed a highly significant increase in NMDAR1 ( $0.462 \pm 0.01$  vs  $0.356 \pm 0.016$ ,  $p=0.0005$ ) as well as the NMDAR2A ( $0.430 \pm 0.023$  vs  $0.378 \pm 0.009$ ,  $p=0.038$ ) and NMDAR2B ( $0.480 \pm 0.01$  vs  $0.430 \pm 0.015$ ,  $p=0.019$ ) mRNA (~~Figs. 9E-J~~).

The immunization effects were specific to these NMDAR mRNAs as no difference in mRNA levels of the trk B receptor were observed in either the dentate granule cell (AAVlac -  $0.310 \pm 0.009$  vs AAVNMDAR1  $0.314 \pm 0.005$ ,  $p=0.706$ ) or CA1 layer ( $0.308 \pm 0.009$  vs  $0.318 \pm 0.006$ ,  $p=0.394$ ) (~~Figs. 9K-L~~). The changes in MK-801 binding, together with the increased NMDA receptor subunit transcripts and NMDA receptor immunoreactivity indicate passage of the antibody across the blood-brain barrier and partial antagonism of the receptor with a compensatory upregulation of NMDA receptors.

#### (iv) Modulation of Krox-24 Transcription Factor

To demonstrate that the antibodies are capable of indirectly modulating events involving the NMDA receptor, an indirect marker of NMDA receptor antagonism was examined. The

expression of Krox-24 protein, a transcription factor whose cortical expression is maintained under tonic NMDA receptor activation, was investigated.

~~Fig. 10A-10H are sections of the cortex of animals.~~ Immunohistochemical images of the cortex of animals were taken demonstrating antibody passage across an intact blood-brain barrier and the reduction of basal levels of Krox-24 protein within the cortex of AAVNMDAR1-vaccinated animals compared to AAVlac-treated, or naïve animals.

High-powered images of Krox-24 immunoreactivity were also recorded.

~~Immunohistochemistry analysis shows reduction of basal levels of Krox-24 protein within the cortex in the AAVNMDAR1-treated (Fig. 10C) compared to AAVlac-treated (Fig. 10B) or naïve animals (Fig. 10A). Insets show high powered images of Krox-24 immunoreactivity.~~ Anti-rat IgG immunohistochemistry used to investigate BBB penetration of IgG under basal conditions showed a weak but increased level of immunoreactivity in the cortex of AAVNMDAR1-treated (Fig. 10F) when compared to AAVlac (Fig. 10E) or naïve (Fig. 10D) animals. IgG penetration was significantly enhanced 90 min following kainate treatment, with specific increases in CA2-CA3 hippocampal regions (arrows, Fig. 10E) compared to the same region under basal conditions (arrows, Fig. 10H). ~~Fig. 10I shows an~~ An immunoblot analysis of CSF sampled from an AAVlac (Lane 1) and AAVNMDAR1 animal (Lane 2) ~~showing~~ showed detection of a 117 ka protein species and breakdown products only from AAVNMDAR1 CSF under basal conditions. Increased levels of these proteins were found 90 min following kainate treatment (Lane 3).

The results demonstrate that antagonism of NMDA receptors using MK-801 leads to a significant decrease in Krox-24 expression. In AAVNMDAR1 (n=6) but not AAVlac (n=5) or naïve control rats (n=4), a  $21 \pm 3.5\%$  ( $p=0.038$ , NMDAR1 vs. lac, Student's t-test) a decrease in Krox-24 protein expression was observed in the cortex (Figs. 10A-C).

To further confirm passage of autoantibodies across the blood-brain barrier, the CSF was screened for the presence of NMDAR1 antibodies as described in Example 3(ii). Low levels of a 117kD protein, consistent with the molecular weight of the native NMDAR 1 receptor subunit and breakdown products similarly recognized by commercial antibodies were detected in the CSF (Fig. 10I). Furthermore, levels of these protein species were increased following kainate treatment. In addition, brain sections of immunized rats were stained with an

anti-rat IgG antibody to look at immunoglobulin transport into brain parenchyma. Under resting conditions, an increase in brain anti-IgG binding was observed in AAVNMDAR1 rats (Figs. 10F) compared to naïve or AAVlac rats (Figs. 10D,E). Following kainate administration, a further increase in the anti-IgG immunoreactivity was apparent in hippocampal sections from AAVNMDAR1 rats (Figs. 10G-H) consistent with the CSF immunoblot result.

(v) Effect of the vaccine on inflammation in the brain

To determine whether there was any inflammatory response, cellular infiltrate or microglial reaction due to vaccination with AAVNMDAR1 vaccination, sections of the brain were analysed. ~~Fig. 11A-11I demonstrate that no~~ No inflammatory responses ~~was~~ were observed in the brain. Similar levels of basal isolectin-B4 (Figs. 11A-C), OX-18 (Figs. 11D-F) and CD8 (Figs. 11G-I) immunoreactivity in the cortex of naïve (Figs. 11A,D,G), AAVlac (Figs. 11B,E,H), AAVNMDAR1 (Figs. C,F,I) suggested no inflammatory responses associated with vaccination. Scale A-1, 500  $\mu$ m. The results showed that brain morphology appeared normal under light microscopy in vaccinated rats, and immunohistochemistry using antibodies to isolectin B4, a microglial marker (Streit, *et al.* (1987) *J. Neurocytol.* 16: 249-60), OX-18, an MHC Class I marker, and CD8, a cytotoxic T-cell marker, showed no differences between AAVNMDAR1, AAVlac and naïve control animals (Figs. 11A-I). The data therefore demonstrates the successful and stable NMDAR1 transgene expression over a period of five months without an inflammatory response.

Please replace the paragraphs starting at line 16 on page 73 and ending at line 21 on page 74 with the following replacement paragraphs:

AAVNMDAR1-vaccinated rats were compared with AAVlac rats to determine whether the immunization was associated with impairment in a spatial maze task (Barnes (1979) *J. Comp. Physiol. Psychol.* 93: 74-104). ~~Fig. 12.~~ Fig. 9 shows the results of AAVNMDAR1 vaccination effects on learning and memory.

Fig. ~~12A~~ 9A is a graph showing errors and latencies recorded on the Barnes Circular Maze from AAVlac-treated rats, and AAVNMDAR1-vaccinated rats. Fig. ~~12B~~ 9B is a graph showing the results from the line crossing and circular track mobility tests. Data represents the number of line crossings in 5 min intervals over 5 successive days in AAVlac-treated, or AAVNMDAR1-vaccinated rats. In the circular track test, the number of completed circuits in successive days for AAVlac-treated and AAVNMDAR1-vaccinated animals are represented. Fig. ~~12C~~ 9C depicts the results from the contextual fear conditioning for AAVlac-treated and AAVNMDAR1-vaccinated animals (\* $p=0.025$ ). Fig. ~~12D~~ 9D depicts the results from the Spontaneous Object Recognition test. The left graph is a comparison within groups of time spent exploring during the sample phase (A1 vs A2) and the choice phase (A3 vs B). The right graph is a comparison between groups of total time spent exploring in sample phase (A1 + A2), choice phase (A3 + B), and the discrimination index (B-A3). (\*  $p=0.041$ ).

Results showed that in the Barnes maze the AAVNMDAR1 rats ( $n=15$ ) had significantly improved performance compared to AAVlac rats ( $n=16$ ) as defined by reduced latencies to enter the escape box (repeated measures ANOVA,  $p=0.043$ , Fig. ~~12A~~ 9A). Improved performance in the Barnes maze may be due to other factors such as increased mobility. To examine increased mobility, the rats were tested on circular track and line crossing mobility paradigms, both of which failed to demonstrate a difference between the groups (repeated measures ANOVA,  $p=0.87$  and  $p=0.32$  respectively, Fig. ~~12B~~ 9B).

NMDA receptor activation has also been demonstrated to be involved in the storage of other forms of memory, such as contextual memory (Kiyama *et al.*, (1998) *J. Neurosci.* 18; 6704-6712 and object recognition memory (Puma *et al.* (1998) *Neurosci. Lett.* 244: 97-100). To assess whether the vaccinated rats had an improved contextual memory, the rats were tested

for their freezing responses. The results demonstrated that AAVNMDAR1-vaccinated rats (n=12) exhibited a stronger freezing response than AAVlac rats (n=10) when placed in an environment in which a mild electric shock had been previously received ( $p=0.025$ , Fig. ~~12C~~ 9C). In addition, AAVNMDAR1 rats (n=19) discriminated and explored a novel object for significantly longer than an object previously encountered compared to AAVlac rats (n=16), ( $p=0.041$ , Fig. ~~12D-9D~~ 9D).

Please replace the paragraphs starting at line 19 on page 75 and ending at line 28 on page 75 with the following two replacement paragraphs:

Fig. ~~13~~ 10 depicts the results vaccination effects on Nociception. The latency for escape responses for the tail immersion test and latency for escape responses or hindpaw licking in the hot plate test for AAVlac (black bars) and AAVNMDAR1 (white bars) animals. Each bar represents the mean  $\pm$  SEM for all animals in that group (\*  $p=0.04$  for tail immersion and  $p=0.02$  for hot plate tests, Student's t-test).

The results demonstrated that following tail immersion, AAVNMDAR1 rats (n=10) showed significantly longer latency to tail-flick than the AAVlac control rats (n=8), ( $p=0.04$ , Fig. ~~13~~ 10). Similarly, in the hot plate assay, the latency of the AAVNMDAR1 rats (n=8) was greater than the AAVlac animals (n=8), ( $p=0.02$ , Fig. ~~13~~ 10).